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ROLE OF GLYCOSAMINOGLYCANS IN THE REGULATION OF T CELL PROLIFERATION INDUCED BY THYMIC STROMA-DERIVED T CELL GROWTH FACTOR[1]

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The present study investigates the regulatory effects of glycosaminoglycans such as heparin and heparan sulfate on T cell proliferation induced by thymic stromal cell monolayer or its derived T cell growth factor (TCGF). A thymic stromal cell clone (MRL104.8a) supported the growth of Ag-specific, IL-2-dependent Th cell clone (9-16) in the absence of Ag and IL-2 by producing a unique TCGF designated as thymic stroma-derived T cell growth factor (TSTGF). The addition of heparin to cultures in which the growth of 9-16 Th cells was otherwise stimulated by the MRL104.8a monolayer or a semipurified sample of the TSTGF resulted in heparin dose-dependent inhibition of 9-16 Th proliferation. The dose of heparin required for inducing 50% reduction of TSTGF-induced proliferation of Th at a given cell number was found to be proportional to the magnitude of the TSTGF added to cultures, suggesting that heparin exerted its inhibitory effect by binding to the TSTGF rather than by acting on Th cells. A similar growth-inhibiting effect of heparin was observed in IL-7-dependent proliferation of pre-B cell line or Th, but not in IL-2-dependent T cell proliferation or IL-3-dependent myeloid cell proliferation. A strong affinity of TSTGF and IL-7 for heparin was confirmed by the fact that both TSTGF and IL-7 adhered to columns of heparin-agarose and were eluted by salt. When various glycosaminoglycans were tested for the heparin-like Th growth-regulatory capacity, heparan sulfate exhibited Th growth-inhibiting ability comparable to that observed for heparin. These results indicate that the activity of thymic and/or bone marrow stroma-derived lymphocyte growth factor (TSTGF/IL-7) but not of Th-producing TCGF (IL-2) is negatively regulated by heparin or heparan sulfate, which would represent major glycosaminoglycans in the extracellular matrix of stromal cells.

The proliferation of eukaryotic cells is regulated by extracellular factors that provide growth-stimulatory and growth-inhibitory signals (1). Over the last decade, a large number of growth-promoting factors and a smaller number of molecules possessing growth-inhibitory activity have been described. Studies have shown that for some of these factors or molecules, their biologic activity might be regulated through its interaction with glycosaminoglycans such as heparin or heparin-like molecules. Thus, along with the evidence that heparin or heparin-like molecules bind to some types of growth factors (2–5), it has been demonstrated that these glycosaminoglycans have a role in stabilizing and synergizing with such growth factors (6–8). In addition, heparin and heparan sulfate have also been shown to inhibit cellular proliferation (9–12) through various mechanisms (13–18).

In contrast to extensive studies concerning the effects of glycosaminoglycans on the proliferation of nonlymphoid cells such as fibroblasts and endothelial cells, the action of glycosaminoglycans on lymphoid cell growth has been poorly investigated. Recently, we have established a thymic stromal cell line and its clones (19) that produce a novel TCGF[3] designated as TSTGF (20, 21). We have also demonstrated that this new TCGF that is distinct from IL-2 or IL-4 can promote the growth of immature thymocytes (22) as well as various Th cell clones (20) without depending on a IL-2- or IL-4-operating autocrine mechanism (23). In considering that bone marrow stroma-derived hematopoietic growth factors have moderate affinity to glycosaminoglycans and that such nature may be involved in hematopoietic cell regulation (24), it would be of great importance to determine whether the mediation of TSTGF activity is also influenced by glycosaminoglycans. Such an investigation could contribute to a better understanding of the roles of glycosaminoglycans in the regulation of intrathymic T cell development.

In the present study we have begun to investigate the interaction of TSTGF with heparin and heparin-like molecules. The results demonstrate that TSTGF is a heparin-binding protein and that a TSTGF-induced T cell proliferation is markedly inhibited by heparin or a heparin-like molecule (heparan sulfate). This contrasted with the failure of heparin to influence Th-derived TCGF (IL-2)-

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[3] Abbreviations used in this paper: TCGF, T cell growth factor; TSTGF, thymic stroma-derived T cell growth factor; KLH, keyhole limpet hemocyanin; HCGF, hematopoietic cell growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; FGF, fibroblast growth factor; ECM, extracellular matrix; SN, supernatant.

induced T cell proliferation, but was similar to heparin- or heparan sulfate-induced inhibition of proliferation of pre-B cell line as stimulated with IL-7. These results indicate that glycosaminoglycans function to negatively regulate the T and/or B cell proliferation that is induced by hematopoietic stromal cell-derived (TSTGF and IL-7) but not by helper T cell-derived (IL-2) lymphocyte growth factor. The results are discussed in the context of the capacity of glycosaminoglycans to compartmentalize stromal cell-derived growth factors as well as their biological relevance to the regulation of lymphocyte proliferation in the hematopoietic/lymphopoietic microenvironment.

MATERIALS AND METHODS

Thymic stroma-derived cell clone. The thymic stroma-derived cell clone (MRL104.8a) has been established in our laboratory from long-term liquid-phase cultures of thymic stromal cells from MRL-1pr/1pr mice (19).

Lymphoid and myeloid cell lines. The following cell lines were used: 9-16 Th clone (25), which is keyhole limpet hemocyanin specific and responds to stimulation with IL-2, IL-4, and TSTGF (19–21); FDC-P2 (26), an IL-3-dependent cell line; PIL-6 (27), an IL-6-dependent plasmacytoma cell line; and DW34 (28), an IL-7-dependent pre-B cell line.

Recombinant cytokines and reagents. Recombinant murine IL-2 was kindly provided by Shionogi Pharmaceutical Co. Ltd. (Osaka, Japan). To obtain rIL-7, genetically engineered CHO cells were established by transfecting the plasmid constructs containing mouse IL-7 and dihydrofolate reductase genes (29) and selecting for cells with high copy number by using methotrexate amplification (30). rIL-7 was used as a form of culture SN of the selected IL-7-transfected CHO cells. Purified murine IL-3 was kindly supplied by Ajinomoto Co. Ltd. (Kanagawa, Japan). rIL-4 and rIL-6 were generous gifts of DNAX Research Institute (Palo Alto, CA). The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): heparin, heparan sulfate, chondroitin sulfate, keratan sulfate, hyaluronic acid, and heparin-agarose.

Partial purification of TSTGF. A semipurified sample of TSTGF was prepared by DEAE-Sephacel chromatography and PBE 94 chromatofocusing as described (21). This semipurified TSTGF sample was free of activities associated with characterized IL and cytokines, including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, and CSF and IFN (19, 21). The titer of TSTGF activity was determined by using 9-16 Th clone as previously described (21) and was expressed in U/ml.

Culture media. Thymic stroma-derived cell clones were maintained in DMEM supplemented with 10% FCS (Hyclone Laboratories, Logan, CT), 5×10^{-5} M 2-ME, and gentamycin (50 μg/ml). This DMEM complete medium was also used for proliferation assays.

Assay system for TSTGF-induced proliferative responses of 9-16 Th clone. Different dilutions of test samples containing TSTGF were cultured with 1×10^4 9-16 Th clone cells in wells of flat-bottomed, 96-well microplates (no. 25860; Corning Glass Works, Corning, NY) at 37°C in a CO_2 incubator (95% air/5% CO_2) for 2 days (20). In some experiments, Th clone (2×10^4/well) was cultured on the monolayer of thymic stromal cell clones (3000 rad, x-irradiated) as previously described (31). The cells were then pulse-labeled with 20 KBq/well of [^{3}H]TdR for 6 h, and the incorporated radioactivity was measured. Results were shown as the mean counts per minute ± SE of triplicate cultures. In experiments assessing the inhibiting activity of heparin for Th proliferation, the proliferation of the 9-16 Th cells was expressed as percentage of control response (100 × cpm in the presence of heparin/cpm in the absence of heparin).

Assay systems for IL-2, IL-3, IL-4, IL-6, and IL-7 activities. Each cytokine activity was assessed by measuring [^{3}H]TdR uptake of IL-2-, IL-4-, and TSTGF-responding 9-16 Th clone (10^4/well) (20), IL-3-dependent FDC-P2 (2×10^4/well) (20), IL-6-dependent PIL-6 (2×10^3/well) (27), or IL-7-dependent DW34 cell line (10^4/well) (28) as previously described.

Heparin-agarose chromatography. A semipurified sample of TSTGF or IL-7-transfected CHO cell culture supernatant was dialyzed against 25 mM imidazole-HCl buffer (pH 7.4), 0.15 M NaCl, and applied to a column (1.5 × 12 cm) of heparin-agarose equilibrated with the same buffer and eluted with a linear gradient of NaCl concentration. Each 5-ml fraction was collected and dialyzed against imidazole-HCl buffer (pH 7.4), 0.15 M NaCl.

RESULTS

Growth promotion of Th clone by the monolayers of thymic stromal cell clones and its inhibition by addition of heparin to cultures. The monolayers of thymic stromal cell clones, MRL104.8a, were prepared in 96-well microculture plates and x-irradiated (3000 rad). Th clone 9-16 (2×10^4/well) was cultured on these irradiated monolayers for 2 days. The data in Table I show that MRL104.8a thymic stromal monolayer is capable of supporting the growth of 9-16 Th clone, which confirms the previous observation that various Th clones can be promoted for their growth by the MRL104.8a monolayer or TSTGF produced in its culture supernatant (19, 31). The proliferation of the 9-16 Th clone on the MRL104.8a stromal monolayers is also shown in phase-contrast microscopic pictures (Fig. 1*A*).

In the above cultures, the effect of heparin on the proliferation of Th clone on thymic stromal monolayers was investigated (Fig. 2). The addition of heparin to proliferating cultures of the 9-16 Th clone induced appreciable inhibition of 9-16 Th growth, although the inhibition

TABLE I

Growth promotion of Th clone on thymic stromal cell monolayers

Experiment	Thymic stromal cell clone[a]	[^{3}H]TdR uptake[b] (cpm)	
		9-16 Th (−)	9-16 Th (+)
1	−	−	1072 (1.07)
	MRL 104.8a	1940 (1.34)	17705 (1.05)
2	−	−	761 (1.07)
	MRL 104.8a	2006 (1.16)	15746 (1.03)

[a] MRL 104.8a monolayers were prepared in 96-well microculture plates and x-irradiated with 3000 rad before culturing with 9–16 Th clone.

[b] Th clone, 9–16 (2×10^4/well) were not or were placed on the monolayers. Two days after coculture, cells were pulse-labeled with [^{3}H]TdR. Incorporated radioactivity was measured and expressed as the geometric mean (SE).

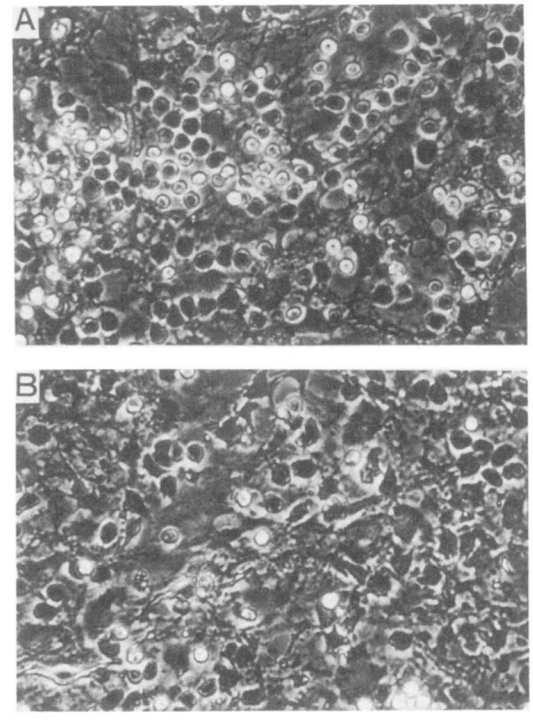

Figure 1. Promotion of Th growth on thymic stromal monolayers and its inhibition by addition of heparin to cultures. Cells of 9-16 Th clone (2×10^5/well) were cultured on the monolayers of MRL104.8a thymic stromal cell clone, which were prepared in 24-well culture plates in the absence (*A*) or presence (*B*) of heparin (100 μg/ml) for 2 days.

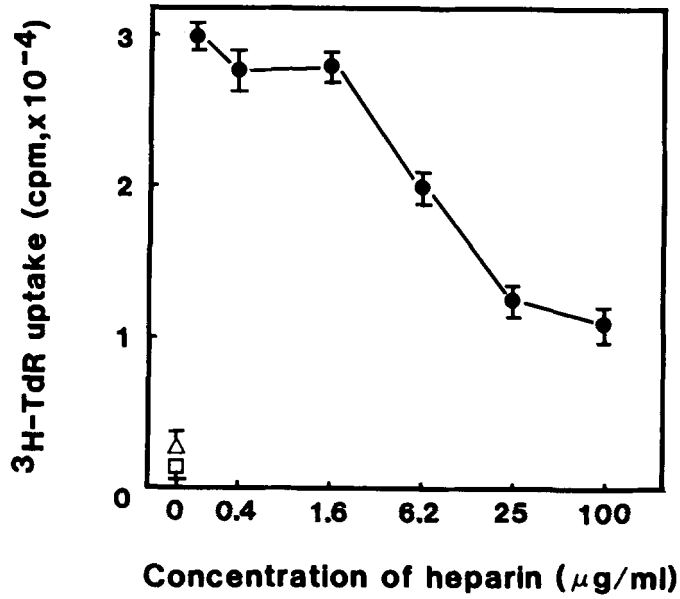

Figure 2. Inhibition of Th growth on thymic stromal monolayers by addition of heparin to culture. 9-16 Th clone (2×10^4/well) was cultured on x-irradiated (3000 rad) monolayers of MRL104.8a in the presence of various concentrations of heparin (●) for 2 days. [^{3}H]TdR uptake by the monolayer alone and 9-16 Th clone alone in the absence of heparin are shown by △ and □, respectively.

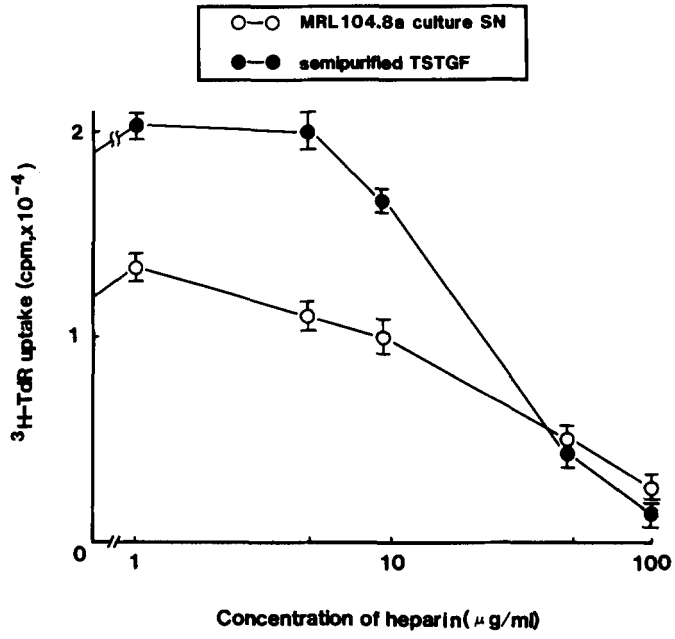

Figure 3. Heparin dose-dependent inhibition of TSTGF-induced Th proliferation. Th clone 9-16 (1×10^4/well) was cultured with MRL104.8a culture SN or a semipurified sample of TSTGF (1 U/ml) for 2 days in the presence of different concentrations of heparin in 96-well microculture plates. [^{3}H]TdR uptake by 9-16 Th clone was expressed as the mean ± SE of triplicate cultures.

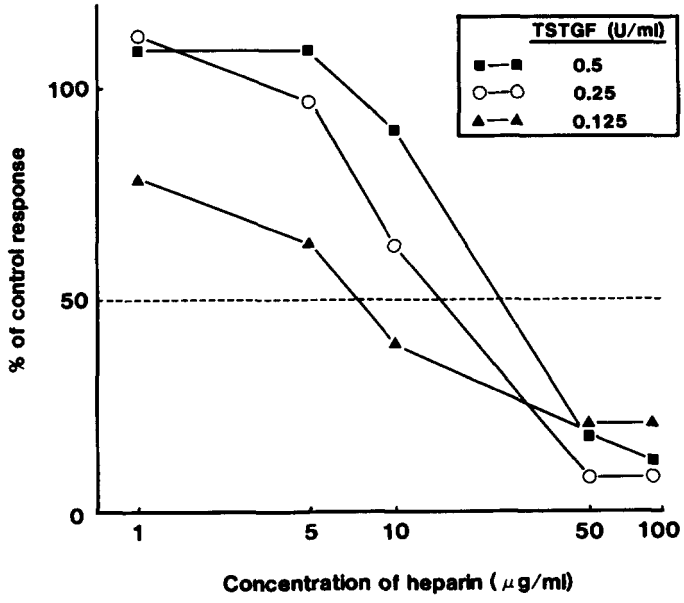

Figure 4. Differential dose requirement of heparin for inhibiting Th proliferation induced by different magnitudes of TSTGF preparation. Th clone 9-16 (1×10^4/well) was cultured with various magnitudes (U/ml) of a semipurified TSTGF sample in the presence of different concentrations of heparin. [^{3}H]TdR uptake by 9-16 Th clone was expressed by percentage control proliferation (response), which represents 100 × (cpm in the presence of heparin/cpm in the absence of heparin). [^{3}H]TdR uptakes (cpm) of 9-16 Th clone that were stimulated with 0.5, 0.25, and 0.125 U/ml of TSTGF in the absence of heparin were 18623 ± 687, 9373 ± 123, and 5870 ± 201, respectively.

was not complete, even in the presence of higher concentrations of heparin. This growth inhibition is also shown in phase-contrast micrographs (Fig. 1*B*). Thus, the growth of Th clone is promoted on the monolayers of a thymic stromal cell clone, and such growth promotion of Th clone is inhibited by heparin.

Heparin inhibits growth promotion of Th clone induced by MRL104.8a-derived factor (TSTGF). We next asked whether heparin-induced inhibition of 9-16 Th proliferation on thymic stromal monolayers is due to blocking the capacity of monolayer-derived TSTGF to promote Th growth-stimulatory activity. The growth of the 9-16 Th clone was stimulated by either MRL104.8a crude culture SN containing TSTGF activity or a semipurified sample of TSTGF (21), and different concentrations of heparin were included in the cultures (Fig. 3). The results illustrate that heparin induces dose-dependent suppression of 9-16 Th proliferation, which is otherwise induced by TSTGF. We have also investigated the heparin dose requirement for inhibiting different magnitudes of TSTGF activities. A constant number of 9-16 Th cells (1×10^4/well) was stimulated with 0.5, 0.25, or 0.125 U/ml TSTGF sample in the presence of different doses of heparin. Figure 4 shows that the dose of heparin required for inhibition of TSTGF activities varied, depending on the magnitude of TSTGF activity used for Th stimulation. That is, approximately four times the dose of heparin was required for the inhibition of 9-16 Th proliferation stimulated by 0.5 U/ml TSTGF, compared with that stimulated by 0.125 U/ml TSTGF, as evident from comparison for 50% inhibition of the 9-16 Th growth. These observations may suggest that heparin exerts its antiproliferative effect on TSTGF-induced Th proliferation by acting on the TSTGF molecules rather than on the 9-16 Th responding cells.

Effect of heparin on various myelo-lymphoid cell proliferation induced by the relevant growth factors. To determine whether heparin-induced inhibition of TSTGF-mediated Th proliferation is observed in other growth factor-dependent myelo-lymphoid cell proliferative systems, the effect of heparin was tested in various cytokine assay systems. These included a) IL-2-responding 9-16 Th, b) IL-3-dependent FDC-P2, c) IL-4-responding 9-16 Th, d) IL-6-dependent PIL-6, and e) IL-7-responding DW34 proliferative responses. The results are sum-

marized in Figure 5 as percentage of control responses of each cytokine-induced proliferation in the presence of different concentrations of heparin. The results illustrate that i) heparin again induced dose-response inhibition of TSTGF-mediated Th proliferation; ii) heparin failed to exert its typical dose-response inhibitory effects on IL-2-, IL-3-, IL-4-, and IL-6-mediated proliferative responses, although IL-4- and IL-6-induced proliferation was inhibited to some extent only at a higher (100 μg/ml) concentration of heparin; and iii) heparin induced dose-response inhibition of IL-7-dependent proliferation similar to that observed for TSTGF. These results indicate that lymphocyte proliferation induced by both bone marrow and thymic stroma-derived cytokines (IL-7 and TSTGF) is inhibited by heparin in a dose-dependent way.

TSTGF and IL-7 adhere to heparin-agarose column. The preceding results, which suggested a possible interaction of heparin with TSTGF, prompted us to investigate whether the TSTGF actually has an affinity for heparin. A heparin-agarose column was prepared, and a semipurified sample of TSTGF was applied to this column to test its binding. Only marginal TSTGF activity was detected in a column-passing fraction (data not shown). TSTGF was eluted with a gradient of increasing salt concentration. The results shown in Figure 6 demonstrate that the TSTGF is bound to a heparin-agarose column at a physiologically ionic strength (0.15 M NaCl) and eluted from the column at 0.5–0.6 M NaCl.

A similar procedure was carried out for IL-7-transfected CHO culture SN. Figure 7 shows that IL-7 activity was also bound to and eluted from a heparin-agarose column at a little higher salt concentration compared to TSTGF. These results indicate that both TSTGF and IL-7 are heparin-binding proteins.

Functional similarity of TSTGF to IL-7. The preceding results suggested that TSTGF is similar or identical to IL-7. Therefore, we examined the functional relationship between these two types of stroma-derived lymphocyte growth factors. The TSTGF and rIL-7 were tested for their abilities to induce the proliferation of responding cell lines used as a target of the respective growth factors as well as alternative responding target cells. The results listed in Table II demonstrate that these two lymphocyte growth factors are capable of supporting the proliferation of both types of indicator cell lines to comparable extents, indicating the functional similarity of TSTGF to IL-7.

Effects of various glycosaminoglycans on TSTGF and IL-7 activities. We finally investigated which types of other glycosaminoglycans are capable of inducing heparin-like growth inhibition of Th and pre-B cell lines. Various glycosaminoglycans were included in TSTGF-induced 9-16 Th or rIL-7-induced DW34 proliferating cultures. The results shown in Figure 8 demonstrate that both growth factor-induced lymphocyte proliferation is inhibited by heparan sulfate, but not by chondroitin sulfate, hyaluronic acid, or keratan sulfate, in a way similar to that of heparin. Thus, heparin and heparan sulfate as a heparin-like molecule inhibit the proliferation of Th and pre-B cell lines induced by TSTGF and rIL-7, respectively.

DISCUSSION

Heparin and heparan sulfate are complex glycosaminoglycans that have several biological effects. Heparin is a potent anticoagulant, and on one hand the basis for some of heparin's action as an anticoagulant is well characterized (32). On the other, the mechanisms by which these glycosaminoglycans influence cell proliferation have been poorly understood. In particular, cellular and molecular mechanisms underlying the antiproliferative capacity of heparin have been analyzed in only a few studies (13–18). These included the inhibition of a critical event in the mid-G1 phase of the cell cycle, which is necessary for subsequent DNA synthesis (17) and the inhibition of a protein kinase C-dependent pathway (18). In this study we have provided an initial description of one mechanism by which heparin and heparin-like molecules may inhibit cell proliferation.

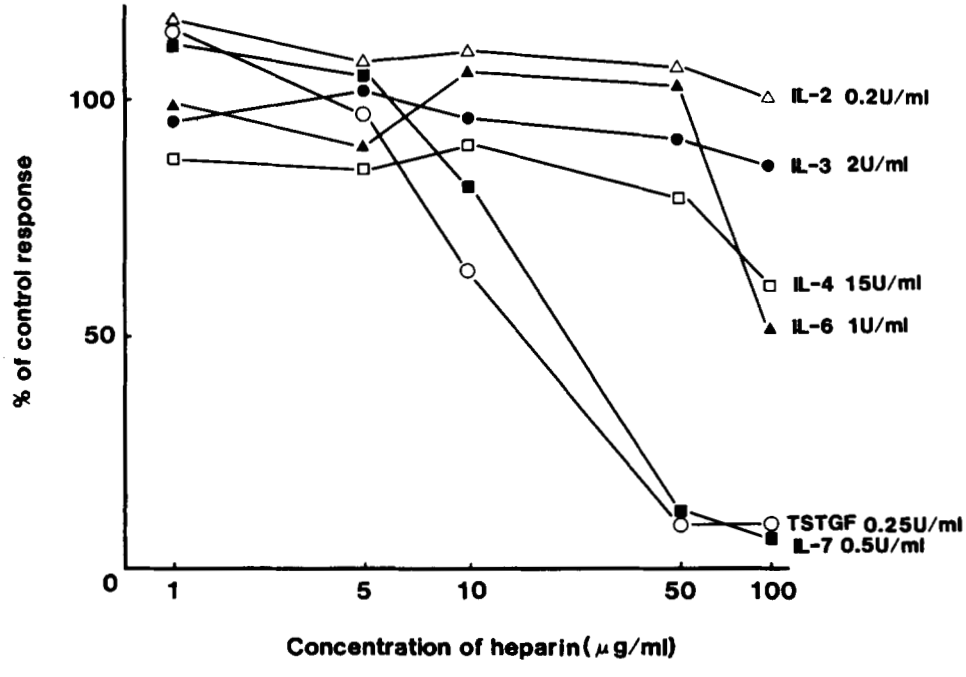

Figure 5. Effect of heparin on myelolymphoid cell proliferation induced by various cytokines. Various cytokine assays were performed by using the corresponding "dependent/responding" cells as described in *Materials and Methods*. The effect of heparin was expressed by percentage control response in the presence of various concentrations of heparin. [^{3}H] TdR uptakes (cpm) by 9-16 Th clone (for TSTGF, IL-2 and IL-4), FDC-P2 (for IL-3), PIL-6 (for IL-6), and DW34 (for IL-7) that were stimulated with the corresponding growth factors in the absence of heparin were 9373 ± 123, 25,366 ± 650, 15,436 ± 847, 8056 ± 187, 58,917 ± 3540, and 49,510 ± 3639, respectively.

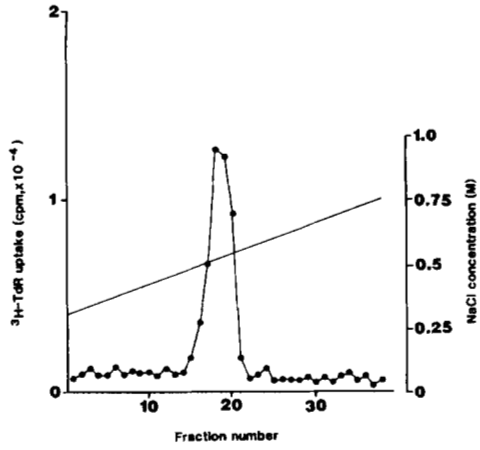

Figure 6. Elution profile of TSTGF activity from heparin-agarose column. A semipurified sample of TSTGF was dialyzed against 25 mM imidazole-HCl buffer (pH 7.4), 0.15 M NaCl, and applied to a heparin-agarose column equilibrated with the same buffer. After the column was washed with the buffer containing 0.3 M NaCl, TSTGF was eluted with a linear gradient of 0.3–0.75 M NaCl (total volume, 200 ml). Fractions of 5 ml each were collected, and after we dialyzed against 25 mM imidazole-HCl, 0.15 M NaCl TSTGF activity in each fraction was measured by using 9-16 Th clone.

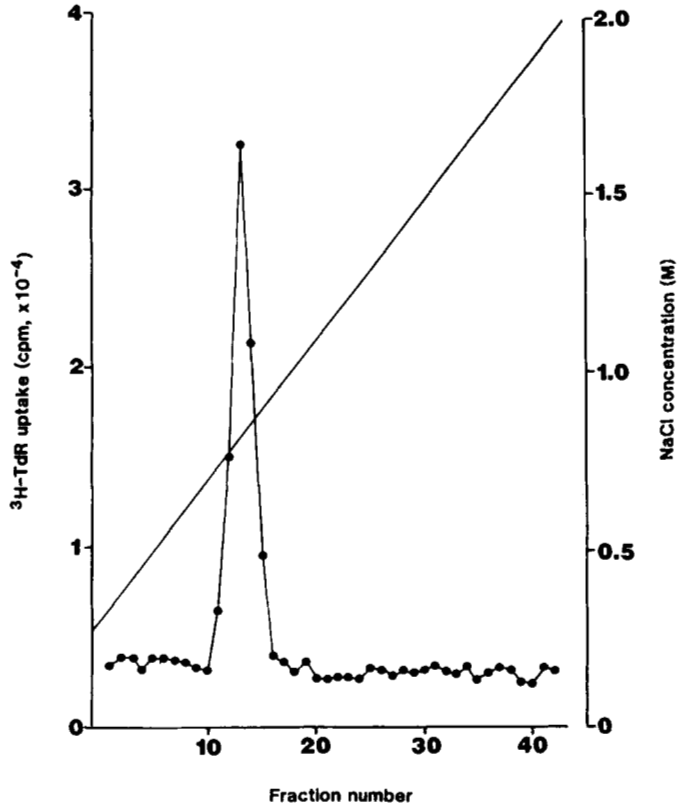

Figure 7. Elution profile of IL-7 activity from heparin-agarose. IL-7-transfected CHO cell culture supernatant was dialyzed and applied to a heparin-agarose column as described in Figure 6. IL-7 was eluted with a linear gradient of 0.3–2.0 M NaCl (total volume, 150 ml). IL-7 activity in each fraction recovered was measured by using a IL-7-dependent pre-B cell line, DW34.

The present results demonstrate that i) heparin inhibits the proliferation of Th cells as induced by thymic stromal cells; ii) heparin also inhibits the Th proliferation induced by its derived TCGF (TSTGF) in a heparin dose-dependent way; iii) TSTGF is a heparin-binding protein; iv) heparin appears to exert its antiproliferative effect by interacting with TSTGF rather than with responding T cells; v) similar antiproliferative action was induced by heparan sulfate; and vi) antiproliferative effects of heparin and heparan sulfate were observed for lymphocyte proliferation induced by bone marrow stroma-derived growth factor (IL-7). Taken with the present observation of the functional similarity of thymic and marrow stroma-derived lymphocyte growth factors (TSTGF and IL-7), our results indicate that heparin/heparan sulfate exerts its regulatory function on the lymphocyte growth induced by stroma-derived (TSTGF/IL-7) but not by Th-derived (IL-2) factors. This study also represents a new mechanism of heparin-induced antiproliferative functions that is distinct from that of its action on responding cells as targets of growth factors (13–18).

TABLE II

Capacities of TSTGF and rIL-7 to support the growth of pre-B cell line and Th clone

Sample Tested (U/ml)	[^{3}H]TdR uptake[a] (cpm, × 10^{-3}) of:			
	DW34		9–16	
	Expt. 1	Exp. 2	Exp. 1	Exp. 2
Medium	2.8 (1.21)	1.1 (1.37)	1.0 (1.30)	2.1 (1.24)
TSTGF (0.5)	31.7 (1.04)	27.6 (1.01)	28.2 (1.20)	34.9 (1.26)
rIL-7 (1.0)	23.8 (1.0)	26.4 (1.13)	25.2 (1.18)	29.4 (1.10)

[a] DW34 pre-B cell line (10^4/well) or 9–16 Th clone (10^4/well) was stimulated with TSTGF or rIL-7 in 96-well microculture plates for 2 days.

It has been reported that heparin and other glycosaminoglycans bind many kinds of glycoproteins, including several growth factors (2–5, 24, 33), as well as cell surface molecules such as CD4 (34). Concerning the interaction of glycosaminoglycans with growth factors, the most extensive studies have been done on the capacity of FGF to bind to heparin and heparan sulfate (2–5). Thus, it has been established that basic FGF as an endothelial cell growth factor has a strong affinity for heparin (3–5) and that this growth factor is synergized (6) or stabilized (7, 8) by heparin. The presence of heparan sulfate as the major glycosaminoglycan in the subendothelial ECM (35) has suggested the positive interaction between glycosaminoglycans and FGF, in that a part of FGF synthesized by endothelial cells, is deposited and sequestered in the subendothelial ECM and may contribute to the induction of cell proliferation (36).

Similar positive interaction of glycosaminoglycans with growth factors have been reported for heparan sulfate on marrow stromal cell surfaces implicated in hematopietic growth factor function (24). In this report, the results demonstrated that HCGF, such as GM-CSF, are synthesized in small amounts by bone marrow stromal cells but remain bound to the stromal cells and/or their ECM via binding to glycosaminoglycans of the ECM. It is possible that the interaction of GM-CSF with the ECM glycosaminoglycans provides the microenvironment in which small amounts of GM-CSF function to produce more effective growth-stimulating effects. This interpretation is in accordance with the observation that hematopoietic progenitor cells can proliferate in close association with bone marrow stromal cells, although biologically active amounts of HCGF are not detected in stromal cell culture supernatants (37–40).

It is apparent from the present study that TSTGF as well as bone marrow stromal cell-derived pre-B cell growth factor (IL-7) bind to heparin and heparin-like molecules. Taken with previous studies dealing with FGF (41, 42), our results permit us to consider the relationship

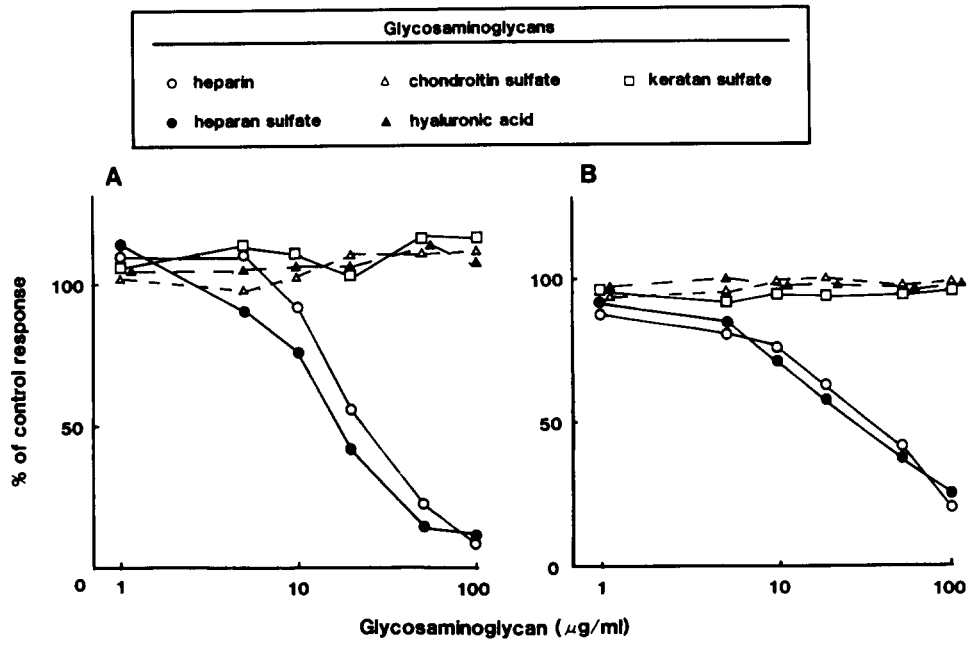

Figure 8. Effects of various glycosaminoglycans on the expression of TSTGF and IL-7 activities. Cells of 9-16 Th clone (1×10^4) (*A*) and DW34 (1×10^4) (*B*) were stimulated with TSTGF (0.5 U/ml) and rIL-7 (1 U/ml), respectively, in the presence of indicated glycosaminoglycans at different concentrations. [^{3}H]TdR uptake (cpm) by 9-16 Th clone and DW34 B cell line in the absence of glycosaminoglycans were 18,623 ± 687 and 39,723 ± 2861, respectively.

between heparin-binding and receptor-binding sites on growth factor molecules. It has been demonstrated that there are multiple heparin-binding sites on FGF, some of which are receptor binding and some which are not (41–43). Although the multiplicity of heparin-binding sites on the TSTGF and/or IL-7 molecules has not been determined in this study, the effective inhibition of TSTGF/IL-7 function by higher doses of soluble heparin/heparin-like molecules indicates that heparin-binding and receptor-binding sites overlap in certain regions of the TSTGF/IL-7 molecule. This contrasts with the finding that heparin did not interfere with the function of IL-3, a heparin-binding protein (33).

The binding nature of heparin/heparin-like molecules observed for TSTGF and IL-7 provides one advantage in that these cytokines can be effectively purified by passing crude culture supernatant through heparin agarose column, as has been reported for other heparin-binding growth factors (44). In fact, such a procedure enabled us to purify the TSTGF, with a more than 1000-fold increase in specific activity, based on protein concentration during the purification process (unpublished observations).

The heparin-binding nature of growth factors should also be discussed in the context of its interaction with the stromal cells by which the relevant hematopoietic growth factors are synthesized. Detailed biochemical analysis of human and mouse bone marrow stroma-derived glycosaminoglycans indicates that heparan sulfate is one of the major constituents (45–47) and functions to absorb HCGF such as GM-CSF (24). Since heparan sulfate has been found to represent a typical glycosaminoglycan constituting the ECM of various types of cells that are of mesodermal origin, it is possible that our thymic stromal cells also generate heparan sulfate on their cell surfaces. If this is the case, our thymic stromal cells might be visualized as concentrating TSTGF on their cell surfaces via heparin-like (heparan sulfate) molecules. The fact that TSTGF activity became detectable in MRL104.8a culture SN several days after the formation of the MRL104.8a monolayer but not 1 to 3 days after the initiation of the MRL104.8a cell culture (19), may be compatible with the above postulation. Thus, it is possible that some part of TSTGF produced by MRL104.8a stromal cells is first consumed in occupying glycosaminoglycan-binding sites on the stromal cells.

There is the fundamental difference in heparin's influences on T cell growth induced by stroma-derived and Th-derived TCGFs. T cell proliferation induced by the Th-derived principal TCGF (IL-2) was not inhibited by higher doses of soluble heparin/heparan sulfates under conditions in which the same concentrations of these glycosaminoglycans elicited potent inhibition of T cell proliferation induced by stroma cell-derived TCGF. Our results illustrate that the growth-promoting capacity of thymic and marrow stroma-derived TCGFs is down-regulated by high doses of soluble heparin/heparin-like substances. Since stromal cells synthesize glycosaminoglycans as well and express them on their surfaces, T cell proliferation on stromal cells might be negatively regulated, depending on the dose relationship between glycosaminoglycans and growth factors. However, it is also possible that under appropriate conditions, glycosaminoglycans on stromal cells function to provide the microenvironment suitable for more efficient growth-promotion by compartmentalizing growth factors synthesized locally by stromal cells. Thus, the interaction of stroma-derived hematopoietic/lymphopoietic growth factors with glycosaminoglycans could represent an important aspect of thymic/marrow microenvironments.

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